ELSEVIER

Neurobiology of Aging 27 (2006) 344–350

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Blueberry supplemented diet reverses age-related decline in hippocampal HSP70 neuroprotection

Rachel L. Galli [a,b], Donna F. Bielinski [a], Aleksandra Szprengiel [a], Barbara Shukitt-Hale [a], James A. Joseph [a,*]

[a] *Neuroscience Laboratory, USDA-ARS Human Nutrition Research Center on Aging at Tufts University, 711 Washington St., Boston, MA 02111, USA*

[b] *Department of Psychology, Simmons College, 300 The Fenway, Boston, MA 02115-5898, USA*

Received 24 September 2004; received in revised form 2 December 2004; accepted 31 January 2005

Abstract

Dietary supplementation with antioxidant rich foods can decrease the level of oxidative stress in brain regions and can ameliorate age-related deficits in neuronal and behavioral functions. We examined whether short-term supplementation with blueberries might enhance the brain's ability to generate a heat shock protein 70 (HSP70) mediated neuroprotective response to stress. Hippocampal (HC) regions from young and old rats fed either a control or a supplemented diet for 10 weeks were subjected to an in vitro inflammatory challenge (LPS) and then examined for levels of HSP70 at various times post LPS (30, 90 and 240 min). While baseline levels of HSP70 did not differ among the various groups compared to young control diet rats, increases in HSP70 protein levels in response to an in vitro LPS challenge were significantly less in old as compared to young control diet rats at the 30, 90 and 240 min time points. However, it appeared that the blueberry diet completely restored the HSP70 response to LPS in the old rats at the 90 and 240 min times. This suggests that a short-term blueberry (BB) intervention may result in improved HSP70-mediated protection against a number of neurodegenerative processes in the brain. Results are discussed in terms of the multiplicity of the effects of the BB supplementation which appear to range from antioxidant/anti-inflammatory activity to signaling.

Keywords: Heat shock protein 70; Polyphenolics; Phytochemicals; Phytonutrients; Free radicals; Oxidative stress; Inflammation; LPS; Aging; Rats

1. Introduction

There is a great deal of research suggesting that one of the most important factors mediating the deleterious effects of aging on behavior and neuronal function is a decreased antioxidant/oxidative/inflammatory stress balance (see [17] for review). The CNS appears to be especially vulnerable to OS effects, partially as a result of additional factors such as increases in the ratio of oxidized to total glutathione [50], significant lipofuscin accumulation [22] with bcl-2 increases [54], increases in membrane lipid peroxidation [72], decreased glutamine synthetase [10], reductions in redox active iron [22,55] and alterations in membrane lipids [14]. Importantly, in addition to these considerations, it has been shown that not only is the CNS particularly vulnerable to OS but this vulnerability increases during aging (see [31,32] for review). With respect to aging, we [8] have shown that senescent rats exhibited significantly greater motor behavioral deficits and decreased tyrosine hydroxylase immunoreactivity (in pars compacta) than young rats following intra-nigral applications of dopamine (DA). Research has also shown that besides the factors discussed above (e.g., reductions in glutathione levels; [50]) OS vulnerability in aging may be the result of several additional factors, such as: (a) changes in the microvasculature [18], (b) increases in oxidized proteins and lipids [18], (c) alterations in the membrane microenvironment, (d) alterations in calcium buffering ability, and (e) differential vulnerability of neurotransmitter receptors [33,34].

Also paralleling the results seen with respect to oxidative stress are increases in sensitivity to inflammatory mediators with aging. For example, Manev and Uz [41] showed that

* Corresponding author. Tel.: +1 617 556 3178; fax: +1 617 556 3222.
E-mail address: james.joseph@tufts.edu (J.A. Joseph).

0197-4580/$ – see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.neurobiolaging.2005.01.017

old rats were more sensitive to kainate-induced excitotoxic brain injuries and enhanced 5-lipoxygenase (5-LOX) expression in limbic structures. Moreover, research has suggested that inflammatory prostaglandins (PG) such as PGE increase in aging, especially in such areas as the hippocampus [13]. In this respect, the PG synthesis pathway appears to be a major source of ROS in several organ systems including brain. [4].

The results of such increases in inflammatory reactions in several pathways involving such factors as cytokines, cycloogenases, prostaglandins, etc. may represent extracellular signals that act in concert to generate additional ROS to induce decrements in neuronal function or glial neuronal interactions [52,56,59,60,66].

It also appears that one important factor in the increased vulnerability to OS and inflammation seen in aging may be decreases in inducible heat shock protein 70 (HSP70; [7,51,62]). Both in vitro and in vivo experiments have demonstrated that increasing the level of inducible HSP70 can protect neuronal cells from a wide range of damaging agents and processes, including heat shock, ischemia, necrosis, toxins, and reactive oxygen species [23,24,38,57]. Moreover, inducible HSP70 works to help damaged proteins regain their correct structure, function and/or location [57,64] and may be neuroprotective.

It might be surmised that the aforementioned reductions in inducible HSP70, possibly along with the other parameters listed above (e.g., alterations in glutathione), contribute to the age-related increases in vulnerability to OS and inflammation that translate into cognitive and motor behavioral deficits. In this respect, studies indicate that oxidative stress [58] and inflammation [25,26] are contributing factors to the behavioral decrements seen in aging.

If this is the case then it might be surmised that interventions designed to reduce the vulnerability of the aged brain to inflammatory and OS insults might translate into reductions in cognitive and motor behavioral deficits. To this end research has shown that nutritional supplements such as Vitamins C or E, garlic [69], herbals (e.g., Ginseng, Ginkgo biloba, Ding lang; see [9]), and dietary fatty acids (reviewed in [70]) have altered these deficits with varying degrees of success. However, we believed that given the considerable antioxidant/anti-inflammatory potential of the fruits and vegetables, they might show considerable efficacy in reducing the deleterious effects of aging on neuronal function and behavior. We utilized fruits and a vegetables identified as being high in antioxidant activity via the oxygen radical absorbance capacity assay (ORAC; e.g., see [67]) and showed that dietary supplementation (for 8 weeks) with spinach, strawberry or blueberry (BB) extracts (2% of an AIN-93 diet; [35]) was effective in reversing age-related deficits in neuronal and behavioral (cognitive, MWM performance) function in aged (19 months) F344 rats. However, only the BB supplemented group exhibited improved performance on tests of motor function. Similar results were seen in a subsequent experiment [71].

Interestingly, analyses of oxidative stress reductions with BB supplementation via dichlorofluorescin diacetate assessments of various brain regions revealed only small but statistically significant effects on DCF [6,35] with no significant changes in glutathione [35]. Thus, it appeared that the beneficial effects of the BB supplementation on oxidative stress appeared to be minimal. These results support those of a recent study [61] that showed that BB supplementation did not alter blood or urine levels of ORAC. If this is the case, the question arises as to other mechanisms that may be involved in the beneficial effects that we have observed from the BB supplementation.

It appears that the multiple bioactive compounds present in fruits such as BBs and their impact on central nervous system function may stem from a combination of factors including anti-inflammatory, anti-viral, anti-microbial, anti-adhesion, and membrane-modulating properties [15,19,21,43,45]. However, we believed that there may be effects on more than one OS/inflammatory parameter. Since it appears, that, as discussed above, inducible HSP70 is a potent antioxidant/anti-inflammatory that is reduced in aging, it may be that BB supplementation may restore the activation of this protein during periods of stress and reduce the vulnerability to these insults and contribute to the beneficial effects observed previously. Therefore, the present study was carried out to determine if BB supplementation in senescent rats would reverse the decrements in LPS-induced HSP70. Note that while hippocampal (HC) responses in LPS-induced HSP70 were assessed in young animals as well, a young BB-supplemented group was not included, since the major focus of this study was to assess effects of BBs on this parameter in old rats as a partial explanation for the beneficial effects of BB supplementation on motor and cognitive function that we had seen previously [35,71].

2. Methods

2.1. Subjects and diet

Thirty male Fischer 344 (F344) rats from the NIA/Harlan colony were housed in pairs on a 12 h light/dark schedule with ad libitum access to food and water. Young rats ($n = 7$) received the control diet, from 4 to 6 months of age. Old rats were randomly assigned to either the control ($n = 7$) or the blueberry supplemented diet ($n = 7$) group and received the diet from ages 19 to 21 months. All subjects were maintained on the diets for 10 weeks prior to in vitro LPS treatment. The rats were weighed weekly. The animals were used in compliance with all applicable laws and regulations as well as principles expressed in the National Institutes of Health, United States Public Health Service Guide for the Care and Use of Laboratory Animals. This study was approved by the Institutional Animal Care and Use Committees of Simmons College and the Human Nutrition Research Center on Aging at Tufts University. The diet was prepared as previously

described [71]. In brief, blueberries were mixed 1:1 with water, homogenized in a blender for 3 min, and centrifuged at 13,000 × *g* for 15 min at 4 °C. The supernatant was frozen and then freeze dried. The resultant crude blueberry extract powder was assayed for antioxidant capacity [63] and incorporated into standard nutritionally complete rat diet at 2% by weight as in Youdim et al. [71] and Joseph et al. [29]. An equivalent amount of corn was added to the control diet.

2.2. In vitro LPS treatment

Brains were dissected on ice and hippocampal regions were individually crosscut (300 mu, McIlwain tissue chopper) and transferred into glass vials containing cold modified Krebs–Ringer buffer (21 mM $NaHCO_3$, 3.4 mM glucose, 1.3 mM NaH_2PO_4, 1 mM EGTA, 0.93 mM $MgCl_2$, 127 mM NaCl, 2.5 mM KCl, bubbled with 95% O_2/5% CO_2). Tissue samples were washed and aliquoted into fresh buffer with either 0 or 10 ug/ml LPS (lipopolysaccharide, Sigma, *E. coli*, serotype 055:B5, TCA extraction), shaken in a 37 °C water bath for 0, 30, 90 and 240 min with fresh buffer +/− LPS replaced at 30-min intervals. Vials were then put on ice, and samples transferred to centrifuge tubes, spun at 10,000 × *g*, 10 min, 4 °C, and dry tissue pellets frozen at −80 °C. This method was adapted from one we have used successfully to assess the effect of oxidative stress on signal transduction in freshly dissected brain tissue [30,35,36].

2.3. Protein preparation

Processing to total cytosolic lysates for western blots began with tissue pellets being resuspended in cold RIPA buffer (1× PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.4) plus protease inhibitors (40 uM PMSF, 2 ug/ml leupeptin, 1 ug/ml pepstatin, 0.1 nM Na vanadate, 1 uM NaF, 5 nM DTT). Samples were vortexed, placed in ice for 30 min, centrifuged at 10,000 × *g* and 4 °C for 10 min, and the supernatant assayed for protein concentration. The resulting total cytosolic lysates were stored at −80 °C for western immunoblots.

2.4. Western immunoblot and statistics

For Western blots, protein samples (60 ug) were run on a 10% SDS-PAGE gel [44]. Control samples included HeLa cell lysate (heat shocked and control), rat brain lysate and HSP70 (SPP-758E, Stressgen). The gel was soaked in protein transfer buffer (TB: 200 mM glycine, 25 mM Tris base, 20% methanol) for 10 min at room temperature, and then blotted onto nitrocellulose using a Hoeffer Transphor Unit. Blots were blocked with TBST (137 mM NaCl, 20 mM Tris, 0.1% Tween-20, pH 7.5) with 5% non-fat dried milk and incubated with primary antibodies (HSC/HSP70, SC1060; cHSC70, SC1059, Santa Cruz; iHSP70, SPA810, Stressgen) in TBST with 5% milk, followed by washing with TBST, and incubation with a horseradish peroxidase-conjugated secondary antibody in TBST with 5% milk. After final washing in TBST, signal was detected using the Renaissance Western Blot Chemiluminescence kit (DuPont NEN). The amount of protein per lane was confirmed using a primary antibody against actin (SC-8432, Santa Cruz). Autogradiograms of the chemiluminescent immunoblots were quantified using a molecular dynamics densitometer and expressed in units of optical density.

Three dependent measures of inducible HSP70 were analyzed: baseline levels (0 ug/ml LPS), stimulated levels (10 ug/ml LPS) and percent change from baseline, which was calculated as the difference from baseline with LPS stimulation expressed as a percent of baseline (10 ug/ml LPS − 0 ug/ml LPS)/0 ug/ml LPS × 100. For each HSP70 measure, and for HSC70 results and actin, two-way between-subjects analysis of variance (ANOVA) models comparing the three groups at the various times (0, 30 and 90 min post LPS) were performed using Systat (SPSS Inc., Chicago, IL) to test for statistical significance at the $p < 0.05$ level. Post-hoc comparisons, to determine differences among the groups, were performed using Fisher's LSD test.

Note that for the 240-min time point a pooled sample was analyzed, since the tissue was used across all the times and was limited for this time. Therefore, the 240 time point was analyzed separately.

3. Results

3.1. Body weights

Analysis of the body weights indicated that the young animals weighed less (388.1 ± 13.8 g) than the BB-supplemented (467.3 ± 12.6 g) or control (469.8 ± 12.7 g) senescent animals contributing to an overall significant difference in the analysis of variance ($F(2,26) = 124.16$, $p < 0.000$). However, as can be seen from the data, there were no differences between the two groups of aged animals ($p > 0.05$ post-hoc Fisher's LSD test).

3.2. Baseline rat HC HSP70 protein levels

As shown in Fig. 1, baseline levels of HSP70 in hippocampal regions across the various times did not differ among the various groups [$F(4,32) = 2.48$, $p = 0.064$]. However, it appeared that the HSP70 was higher at the 30 and 90 min times than at the 0 time [$F(2,32) = 11.73$, $p < 0.001$].

3.3. Time course of HSP70 induction in rat HC following LPS challenge

The results of the putative age by diet-related differences in the time course of the hippocampal HSP70 response to LPS indicated that these differences did not appear until 90 min following LPS exposure (Fig. 2) [group effect, $F(2,32) = 8.47$, $p < 0.01$; time effect, $F(2,32) = 48.52$,

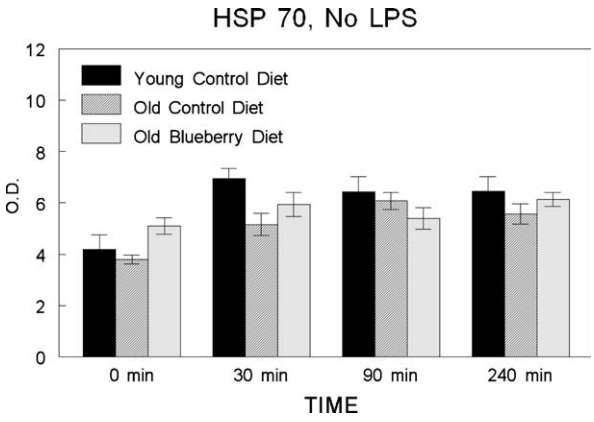

Fig. 1. Baseline levels of heat shock protein 70 (HSP70) at various incubation times (0, 30, 90 and 240 min) in hippocampal regions of young rats fed a control diet, old rats fed a control diet, and old rats fed a blueberry supplemented diet for 10 weeks.

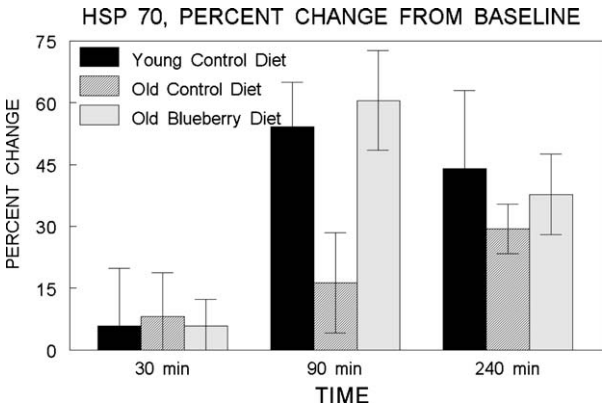

Fig. 3. Percent increase in hippocampal HSP70 levels from baseline in response to an in vitro LPS challenge at various times post LPS (30, 90 and 240 min) of young rats fed a control diet, old rats fed a control diet, and old rats fed a blueberry supplemented diet for 10 weeks.

$p < 0.001$]. Thus, no differential effects of LPS exposure on HSP70 activation were seen among the groups at the 30 min time point following LPS treatment.

Post-hoc testing showed that following 90 min of in vitro exposure to 10 ug/ml LPS, increases in HSP70 levels were significantly lower in old control diet rats as compared to young control diet rats in brain hippocampal regions ($p < 0.001$). Hippocampal regions from BB-supplemented old rats produced HSP70 levels that were higher than old control diet rats ($p < 0.05$) and no different from the young rats ($p > 0.05$). These group differences were maintained at 240 min after LPS treatment [$F(2,6) = 8.95$, $p < 0.05$] (Fig. 2).

When examining percent change from baseline, i.e., no LPS induced HSP70 versus LPS induced HSP70 at each time, the group differences were primarily seen at 90 min [$F(2,12) = 4.17$, $p < 0.05$] (Fig. 3). Again, the percent change in senescent rats fed the control diet was lower than that of young control diet rats ($p < 0.05$), while the BB-supplemented old rats had a percent increase that was not different than the young rats ($p = 0.71$), yet higher than age-matched controls ($p < 0.05$). However, at the 240 min time point, there were no differences between the groups on the effects of LPS on HSP70 induction [$F(2,6) = 0.33$, $p = 0.73$] (Fig. 3). As is also shown in Fig. 3, the non-significant differences among the groups at 240 min appears to be the result of reductions in LPS-induced HSP70 in the young and BB-supplemented groups and a slight but non-significant increase in the old control group, rather than a slower rise in the induction of HSP70 in this group.

Finally, there were no differences in HSC70 or actin as a function of age or diet as a function of the length of the LPS exposure.

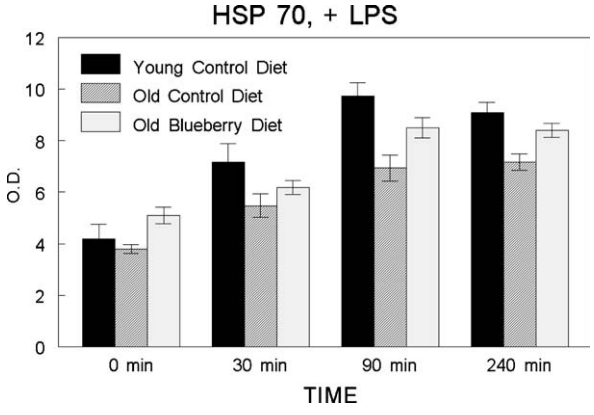

Fig. 2. Stimulated levels of heat shock protein 70 (HSP70) at various times following incubation with LPS (0, 30, 90 and 240 min) in hippocampal regions of young rats fed a control diet, old rats fed a control diet, and old rats fed a blueberry supplemented diet for 10 weeks.

4. Discussion

As mentioned in the introduction, with age there are increased levels of, and reduced protective mechanisms against, cellular stresses such as inflammation and oxidative stress [5,46,48]. One of the most important of these is HSP70. The induction of HSP70 in response to heat stress has been demonstrated in a wide range of studies to protect cells from subsequent damage. HSP70 has been described as a molecular chaperone that works in part to restore damaged proteins to their original structure and function [57,65]. The amount of protection afforded has been shown to be proportional to the level of HSP70 induction [1–3,68]. Within the brain, HSP70 mediated cell survival is also affected by cell type, brain region, and age-dependent differences in the time course of the response to hyperthermia [47,51]. In general, mammals become less tolerant to heat stress with old age, as evidenced by the higher rate of death by hyperthermia in old rats [28] and humans [16]. A reduction in the size of the HSP70 response [49,27] may play a role in the increased vulnerability to stressors that occurs with age, and as mentioned in the Introduction, both oxidative stress [58] and inflammation [25,26] are contributing factors to the behavioral decrements seen in aging.

In this regard, the present findings show that there were no differences among the groups in basal HSP70. However, it also appeared that acute LPS-induced HSP70 production was significantly decreased at the 90 and 240 min post LPS times in the senescent control rats as compared to the aged BB-supplemented and young control rats. The finding showing that there were age differences in the response at both of these later time points suggest that the differences observed were not the result of age differences in amount of time required to mount a full HSP70 response as compared to the age differences in overall size of response.

Instead the results showed that short-term nutritional intervention with BB supplementation increased HSP70 mediated protection in the hippocampal regions of old rats against a stressor such as LPS, and this difference is maximized at the 90 and 240 post LPS time points (Fig. 2). These findings suggested that short-term BB supplementation restored the ability of the aged hippocampal cells to respond to an inflammatory challenge with a large production of inducible HSP70.

Thus, it might be postulated that the increases in stress induced HSP70 activity in the BB-supplemented aged rats could play a role in shifting the balance between pro- and anti-oxidant forces [5] to a response that was similar to those seen in the young animals. As HSP70 is synthesized in response to a host of damaging stimuli, these findings suggest that the blueberry supplemented diet may result in improved protection against a number of age-related neurodegenerative processes in the brain.

Previous studies have suggested that although age-related changes in hippocampal function impair spatial learning and memory in rats and humans [20,39,42,53], the improved protection may combine with other factors such as increases in hippocampal neurogenesis and signaling [12] that ultimately translate into improved behavioral performance. In this regard, as mentioned in Section 1, BB supplementation significantly improved performance on tests of spatial learning and memory [9,35,36,71] in the sensecent rats.

The mechanisms involved in the enhanced HSP70 response to LPS in the hippocampal tissue also may involve altered signaling, since we have recently shown that BB supplementation enhanced hippocampal ERK activity in APP/PS-1 mice [29]. Recent work also indicates that hyperthermic-induced stress decreased hippocampal ERK activity and increased HSP70 activity. There is also work to show that curcumin, a potent spice with antioxidant/anti-inflammatory activity, antagonizes the effects of LPS by down-regulating the Janus (JAK) kinase-STAT inflammatory pathway [37]. Results from several experiments suggest that the LPS involves activation of microglia and subsequent induction of glutamate. These would ultimately affect neuronal cells in the hippocampus (e.g., see [40]). Further analyses of the putative role of BB supplementation in altering signaling in LPS-induced HSP70 activity will be the subject of future experiments, as will determination of the particular cell type involved in these activations.

Finally, it should be mentioned that although the BB-supplemented animals did not show any loss of weight as compared to the rats fed the control diet, the effects seen here with respect to LPS-induced enhancement of HSP70 are similar to those seen previously with long-term caloric restriction (CR) (see [11] for review). It should also be noted that transgenic mouse models of Alzheimer disease (AD) respond to food restriction with increased resistance to AD-related synaptic changes [74]. This protection correlates with enhanced HSP70 production in cortical synaptosomes and with HSP70's effect on calcium regulation, membrane potential, and resistance to oxidative stress [24,62,73]. This neuroprotection is postulated to be a critical factor in the beneficial effect of caloric restriction on both life expectancy and animal models of Alzheimer disease [24,73].

5. Conflict of interest statement

The laboratory of James Joseph receives funding from the Wild Blueberry Association on North America (WBANA) and the U.S. Highbush Blueberry Council (USHBC). Simmons College Fund for Research provided support to R. Galli.

References

[1] Amin V, Cumming DVE, Coffin RS, Latchman DS. The degree of protection provided to neuronal cells by pre-conditioning stress correlates with the amount of heat shock protein 70 it induces and not with the similarity of the subsequent stress. Neurosci Lett 1995;200:85–8.
[2] Amin V, Cumming DVE, Latchman DS. Over-expression of heat shock protein 70 protects neuronal cells against both thermal and ischaemic stress but with difference efficiencies. Neurosci Lett 1996;206:45–8.
[3] Armstrong JN, Plumier JC, Robertson HA, Currie RW. The inducible 70,000 molecular/weight heat shock protein is expressed in the degenerating dentate hilus and piriform cortex after systemic administration of kainic acid in the rat. Neuroscience 1996;74(3):685–93.
[4] Baek BS, Kim JW, Lee JH, Kwon HJ, Kim ND, Kang HS, et al. Age-related increase of brain cyclooxygenase activity and dietary modulation of oxidative status. J Gerontol A Biol Sci Med Sci 2001;56:B426–31.
[5] Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 1997;272(33):20313–6.
[6] Bickford PC, Gould T, Briederick L, Chadman K, Pollock A, Young D, et al. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res 2000;866(1–2):211–7.
[7] Blake MJ, Fargnoli J, Gershon D, Holbrook NJ. Concomitant decline in heat-induced hyperthermia and HSP70 mRNA expression in aged rats. Am J Physiol 1991;260:R663–7.
[8] Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. Dopamine neurotoxicity: age-dependent behavioral and histological effects. Neurobiol Aging 2003;24:697–706.
[9] Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. Neurobehavioral aspects of antioxidants in aging. Int J Dev Neurosci 2000;18:367–81.
[10] Carney JM, Smith CD, Carney AM, Butterfield DA. Aging- and oxygen-induced modifications in brain biochemistry and behavior. Ann N Y Acad Sci 1994;738:44–53.

[11] Casadesus G, Shukitt-Hale B, Joseph JA. Qualitative versus quantitative caloric intake: are they equivalent paths to successful aging? Neurobiol Aging 2002;23:747–69.
[12] Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee H-G, Smith MA, et al. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 2004;7:309–16.
[13] Casolini P, Catalani A, Zuena AR, Angelucci L. Inhibition of COX-2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat. J Neurosci Res 2002;68:337–43.
[14] Denisova NA, Erat SA, Kelly JF, Roth GS. Differential effect of aging on cholesterol modulation of carbachol stimulated low-Km GTPase in striatal synaptosomes. Exp Gerontol 1998;33:249–65.
[15] Eastwood MA. Interaction of dietary antioxidants in vivo: how fruits and vegetables prevent disease. QJM 1999;92(9):527–30.
[16] Ellis FP. Mortality from heat illness and heat-aggravated illness in United States. Environ Res 1972;5:1–58.
[17] Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 1999;222:236–45.
[18] Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 2002;23:795–807.
[19] Fotsis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H, et al. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 1997;57(14):2916–21.
[20] Gallagher M. Animal models of memory impairment. Phil Trans R Soc Lond 1997;352:1711–7.
[21] Gerritsen ME. Flavonoid inhibitors of cytokine induced gene expression. In: Manthey J, Buslig B, editors. Flavonoids in the living system. 1st ed. New York: Plenum Press; 1998. p. 183–90.
[22] Gilissen EP, Jacobs RE, Allman JM. Magnetic resonance microscopy of iron in the basal forebrain cholinergic structures of the aged mouse lemur. J Neurol Sci 1999;168:21–7.
[23] Gordon SA, Hoffman RA, Simmons RL, Ford HR. Induction of heat shock protein 70 protects thymocytes against radiation-induced apoptosis. Arch Surg 1997;132(12):1277–82.
[24] Guo ZH, Mattson MP. In vivo 2-deoxyglucose administration preserves glucose and glutamate transport and mitochondrial function in cortical synaptic terminals after exposure to amyloid B-peptide and iron. Exp Neurol 2000;166:173–9.
[25] Hauss-Wegrzyniak B, Vannucchi MG, Wenk GL. Behavioral and ultrastructural changes induced by chronic neuroinflammation in young rats. Brain Res 2000;859:157–66.
[26] Hauss-Wegrzyniak B, Vraniak P, Wenk GL. The effects of a novel NSAID on chronic neuroinflammation are age dependent. Neurobiol Aging 1999;20:305–13.
[27] Helfert RH, Glatz III FR, Wilson TS, Ramkumar V, Hughes LF. Hsp70 in the inferior colliculus of Fischer-344 rats: effects of age and acoustic stress. Hear Res 2002;170(1–2):155–65.
[28] Hoffman-Goetz L, Keir R. Fever and survival in aged mice after endotoxin challenge. J Gerontol 1985;40:15–22.
[29] Joseph JA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. Nutr Neurosci 2003;6:153–62.
[30] Joseph JA, Dalton TK, Roth GS, Hunt WA. Alterations in muscarinic control of striatal dopaminergic autorectoprs in senescence: a deficit at the ligand-muscarinic recptor interface? Brain Res 1988;454(1–2):149–55.
[31] Joseph JA, Denisova N, Fisher D, Bickford P, Prior R, Cao G. Age-related neuro-degeneration and oxidative stress: putative nutritional intervention. Neurol Clin 1998;16:747–55.
[32] Joseph JA, Denisova NA, Fisher D, Shukitt-Hale B, Bickford P, Prior R, et al. Membrane and receptor modifications of oxidative stress vulnerability in aging: nutritional considerations. Ann N Y Acad Sci 1998;854:268–76.
[33] Joseph JA, Fishe DR. Muscarinic receptor subtype determines vulnerability to amyloid beta toxicity in transfected COS-7 cells. J Alzheimers Dis 2003;5:197–208.
[34] Joseph JA, Fisher DR, Strain J. Muscarinic receptor subtype determines vulnerability to oxidative stress in COS-7 cells. Free Radic Biol Med 2002;32:153–61.
[35] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 1999;19(18):8114–21.
[36] Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, et al. Long-term dietary strawberry, spinach, or Vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 1998; 18(19):8047–55.
[37] Kim HY, Park EJ, Joe EH, Jou I. Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. Immunology 2003;171:6072–9.
[38] Lowenstein DH, Chan PH, Miles MF. The stress protein response in cultured neurons: characterization and evidence for a protective role in excitotoxity. Neuron 1991;7:1053–60.
[39] Lynch MA, Voss KL, Gower AJ. Impaired spatial memory in aged rats is associated with alterations in inositol phospholipid metabolism. NeuroReport 1994;5:1493–7.
[40] Ma XC, Gottschall PE, Chen LT, Wiranowska M, Phelps CP. Role and mechanisms of interleukin-1 in the modulation of neurotoxicity. Neuroimmunomodulation 2002–2003;10:199–207.
[41] Manev H, Uz T. Primary cultures of rat cerebellar granule cells as a model to study neuronal 5-lipoxygenase and FLAP gene expression. Ann N Y Acad Sci 1999;890:183–90.
[42] Markowska AL, Stone WS, Ingram DK, Reynolds J, Gold PE, Conti LH, et al. Individual differences in aging: behavioral and neurobiological correlates. Neurobiol Aging 1989;10:31–43.
[43] Martin A, Prior R, Shukitt-Hale B, Cao G, Joseph JA. Effects of fruits, vegetables, or Vitamin E-rich diet on Vitamins E and C distribution in peripheral and brain tissues: implications for brain function. J Gerontol 2000;55A(3):144–51.
[44] Mendelson KG, Contois LR, Tevosian SG, Davis RJ, Paulson KE. Independent regulation of JNK/p38 mitogen activated protein kinases by metabolic oxidative stress in the liver. Proc Natl Acad Sci USA 1996;93:12908–13.
[45] Middleton Jr E. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998;439:175–82.
[46] Morgan TE, Xie Z, Goldsmith S, Yoshida T, Lanzrein AS, Stone D, et al. The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. Neuroscience 1999; 89(3):687–99.
[47] Morrison-Bogorad M, Pardue S, McIntire DD, Miller EK. Cell size and the heat-shock response in rat brain. J Neurochem 1994;63(3):857–67.
[48] Murray CA, McGahon B, McBennett S, Lynch MA. Interleukin-1 inhibits glutamate release in hippocampus of young, but not aged, rats. Neurobiol Aging 1997;18(3):343–8.
[49] Njemini R, Demanet C, Mets T. Inflammatory status as an important determinant of heat shock protein 70 serum concentrations during aging. Biogerontology 2004;5(1):31–8;
Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;76:139–62.
[50] Olanow CW. An introduction to the free radical hypothesis in Parkinson's disease. Ann Neurol 1992;32:S2–9.
[51] Pardue S, Groshan K, Raese JD, Morrison-Bogorad M. HSP70 mRNA induction is reduced in neurons of aged rat hippocampus after thermal stress. Neurobiol Aging 1992;13(6):661–72.
[52] Rosenman SJ, Shrikant P, Dubb L, Benveniste EN, Ransohoff RM. Cytokine induced expression of vascular cell adhesion molecule-

1 (VCAM-1) by astrocytes and astrocytoma cell lines. J Immunol 1995;154:1888–99.
[53] Rosenzweig ES, Barnes CA. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Progr Neurobiol 2003;69(3):143–79.
[54] Sadoul R. Bcl-2 family members in the development and degenerative pathologies of the nervous system. Cell Death Differ 1998;5:805–15.
[55] Savory J, Rao JK, Huang Y, Letada PR, Herman MM. Age-related hippocampal changes in Bcl-2:Bax ratio, oxidative stress, redox-active iron and apoptosis associated with aluminum-induced neurodegeneration: increased susceptibility with aging. Neurotoxicology 1999;20:805–17.
[56] Schipper HM. Astrocytes, brain aging, and neurodegeneration. Neurobiol Aging 1996;17:467–80.
[57] Sharp FR, Massa SM, Swanson RA. Heat-shock protein protection. TINS 1999;22(3):97–9.
[58] Shukitt-Hale B. The effects of aging and oxidative stress on psychomotor and cognitive behavior. Age 1999;22:9–17.
[59] Steffen B, Breier G, Butcher E, Schulz M, Engelhardt B. VCAM-1, and MAdCAM-1 are expressed on choroid plexus epithelium but not endothelium and mediate binding of lymphocytes in vitro. Am J Pathol 1996;148:1819–38.
[60] Stella N, Estelles A, Siciliano J, Tence M, Desagher S, Piomelli D, et al. Interleukin-1 enhances the ATP-evoked release of arachidonic acid from mouse astrocytes. J Neurosci 1997;17:2939–46.
[61] Sweeney MI, Kalt W, MacKinnon SL, Ashby J, Gottschall-Pass KT. Feeding rats diets enriched in lowbush blueberries for six weeks decreases ischemia-induced brain damage. Nutr Neurosci 2002;5(6):427–31.
[62] Walters TJ, Ryan KL, Mason PA. Regional distribution of Hsp70 in the CNS of young and old food-restricted rats following hyperthermia. Brain Res Bull 2001;55(3):367–74.
[63] Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agric Food Chem 1996;44(3):701–5.
[64] Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev 1992;72(4):1063–81.
[65] Welch WJ. Heat shock proteins functioning as molecular chaperones: their roles in normal stressed cells. Phil Trans R Soc Lond 1993;339:327–33.
[66] Woodroofe M. Cytokine production in the central nervous system. Neurology 1995;45:S6–10.
[67] Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem 2004;52:4026–37.
[68] Yenari MA, Fink SL, Sun GH, Chang LK, Patel MK, Kunis DM, et al. Gene therapy with HSP72 is neuroprotective in rat models of stroke and epilepsy. Ann Neurol 1998;44(4):584–91.
[69] Youdim KA, Joseph JA. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. Free Radic Biol Med 2001;30:583–94.
[70] Youdim KA, Martin A, Joseph JA. Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 2000;18:383–99.
[71] Youdim KA, Shukitt-Hale B, Martin A, Wang H, Denisova N, Bickford PC, et al. Short-term dietary supplementation of blueberry polyphenolics: beneficial effects on aging brain, performance and peripheral tissue. Nutr Neurosci 2000;3:383–97.
[72] Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994;76:139–62.
[73] Yu ZF, Mattson MP. Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. J Neurosci Res 1999;57:830–9.
[74] Zhu H, Guo Q, Mattson MP. Dietary restriction protects hippocampal neurons against the death-promoting action of a presenilin-1 mutation. Brain Res 1999;842:224–9.